DECREASED GLUCOSE TOLERANCE IN TWO MEN DURING EXPERIMENTAL COPPER DEPLETION

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ABSTRACT

The daily dietary copper requirement of two healthy men was found to exceed 0.78 mg in a depletion experiment. This amount of dietary copper is similar to that in some contemporary diets. Intravenous glucose tolerance tests were done during the control, depletion and repletion phases of the study. In response to copper depletion the ability of these men to clear a glucose load decreased while hematological indices were unchanged. Glucose clearance improved upon repletion with 6 mg of copper per day; some points on these final curves were lower than corresponding points on curves before depletion. Glucose clearance may be a more sensitive index of copper nutriture than are changes in hematology, plasma copper, ceruloplasmin or cholesterol or erythrocyte superoxide dismutase. The results of this experiment are consonant with experiments with animals and may be of importance in the etiology or pathophysiology of mild diabetes mellitus.

INTRODUCTION

The origin of diabetes mellitus generally remains obscure. Various names have been assigned to the two idiopathic forms of the disease. A more severe form tends to occur early in life and often is associated with severe insulin deficiency. The less severe form is more common, tends to occur later in life and, in some cases, is associated with decreased cellular response to circulating insulin (1,2). Measurement of glucose in blood after a loading dose is helpful in diagnosis of both forms of diabetes (1,2).

Trace elements are involved in the metabolism of glucose and may be important in either the etiology or pathophysiology of some cases of diabetes. Manganese deficiency in Guinea pigs produces glucose intolerance (3); a man with diabetes was treated successfully with a folk remedy the active principle of which was found to be manganese (4,5). Chromium has been studied more extensively than the other trace elements in its relationship to diabetes (6,7). Data from animals and humans support the belief that chromium deficiency produces glucose intolerance. Zinc is thought to be involved in the storage and secretion of insulin (8,9). Pharmacological doses of selenium ameliorated the glucose intolerance induced in rats by toxicologic doses of cadmium (10).

Abnormal glucose metabolism in rats deficient in copper was discovered 50 years ago by Keil and Nelson (11). In modern terminology this abnormality is called glucose intolerance or impaired glucose tolerance. There is renewed interest in this phenomenon (12,13); the concentration of glycosylated hemoglobin is increased in rats deficient in copper (14).

We are engaged in long-term research to determine nutritional requirements. We present evidence that the dietary requirement for copper of two men exceeded 0.78 mg per day. The principal manifestation of impaired nutriture was an impairment of glucose tolerance that returned to normal upon oral repletion with copper sulfate.

Case-reports. Two healthy men[1] aged 21 and 30 years consented to participate in the study after extensive explanation of its objectives and potential hazards. Their histories and physical examinations were unremarkable. Neither was diabetic by any criterion and neither had a family history of diabetes. Neither was obese; they measured 175 and 177 cm and weighed 86.6 and 69.0 kg, respectively. Pulse, blood pressure, respiratory rate and body temperatures were normal. Hemoglobin, hematocrit, erythrocyte count and indices, neutrophil count, plasma ferritin and iron, total iron binding capacity and transferrin were normal as were more than 50 indices of nutritional status and general health.

METHODS

This research was carried out according to the principles of the Declaration of Helsinki, informed consent was obtained, and the Institutional Review Board of the University of North Dakota approved the study.

The study was conducted in a metabolic ward; the volunteers were under constant supervision. They were instructed in the various procedures and in the rules of acceptable behavior. Smoking was prohibited. Meals made from the foods in Table 1 were eaten completely; no other foods were permitted. The environment has been described (15,16).

The volunteers ate the diet in Table I repetitively. That is, after each three days, they began the cycle again for a total of 175 or 150 days. The daily diet was calculated to provide 2500 kcal. from conventional foods with additional energy for weight maintainance provided by a commercial[2] glucose polymer. The volunteers received 750 or 250 kcal. of this polymer during all but a short period at the beginning of the study. The diet contained

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[1]When data are presented in pairs, the data are on volunteer 2082 and 2083, respectively.

[2]Polycose, Ross Laboratories, Columbus, Ohio 43216. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

amounts of essential nutrients similar to the Recommended Dietary Allowances (17); calcium, copper and zinc were exceptions. Measurement of these elements in dietary homogenates by atomic absorption spectrometry revealed daily amounts to be 780, 0.78 and 11.4 mg, respectively. The diet was supplemented daily with 180 mg calcium and 8.5 mg zinc (both as gluconate).

Table I. Composition of the 2500 kcal. Diet*, gram

	Day 1		Day 2		Day 3	
BREAKFAST	Grapefruit juice	200	Orange juice	200	Pineapple juice	250
	Milk	100	Hot corn cereal	155	Jelly	50
	Cornflakes	23	Jelly	25	Bread	40
	Jelly	25	Bread	20	Margarine	22.5
	Bread	20	Margarine	10		
	Margarine	10				
LUNCH	Pineapple juice	250	Grape juice	250	Grapefruit juice	200
	Applesauce	250	Hamburger cheese casserole	191	Cornbread	131
	Cheese casserole	177	Cornbread	131	Oven baked chicken breast	81
	Green beans	60	Jelly	25	Green beans	50
	Jelly	25	Margarine	15	Sugar cookie	44
	Sugar cookie	22			Jelly	25
	Bread	20			Margarine	15
	Margarine	12.5				
DINNER	Orange juice	200	Grapefruit juice	250	Grape juice	250
	Baked pike	135	Crispy chicken	100	Citrus salad	171
	Cheesecake	90	Carrots	50	Hot tuna sandwich	100
	Sugar cookie	33	Jelly	25		
	Bread	20	Sugar cookie	22		
	Jelly	18	Bread	20		
	Margarine	10	Butter	12.5		
SNACK	Grape juice	200	Pineapple juice	250	Orange juice	200
	Pears	75	Sugar cookie	22	Applesauce	200
	Cheese cracker	33			Sugar cookie	44

*Percent of energy: carbohydrate, 63; protein, 8; fat, 29.

The study had three phases. A control phase, during which the diet was supplemented daily with an extra 0.5 mg of copper, lasted 30 days. During the control phase the subjects became accustomed to the diet and the environment. The depletion phase lasted 120 or 90 days; no copper supplement was given. The repletion phase lasted 30 days; the diet was supplemented to 6 mg of copper daily.

Approximately fifty measurements of nutritional status and general health were made at regular intervals throughout the study. Hematology and assessments of iron nutriture were done using standard methods (16). Copper in diets and plasma was measured by atomic aosorption spectrometry (15) after collection, storage and processing of samples in equipment known to be free of contamination. Superoxide dismutase (E.C.1.15.1.1) activity was measured in erythrocytes according to Winterbourn et al. (18). Ceruloplasmin was measured by radial immunodiffusion (19). Intravenous glucose tolerance tests were done at the end of each phase of the study after a 9 hr. fast before 8 a.m. by infusing 0.5 g of glucose (as a 25% solution) per kg of body weight in a 3-5 minute period and flushing the indwelling catheter with normal saline (20). Glucose and insulin in plasma were measured with glucose oxidase (21) and radioimmunoassay (22). Cholesterol in plasma lipoproteins was measured by standard methods (23).

Maximal exercise capacity of the volunteers was measured before depletion on a bicycle ergometer using a progressive test similar to the protocol of Astrand and Rhyming (24) with continuous electrocardiography. During the depletion phase, exercise was restricted to an ergometer load which produced 50% of maximal oxygen consumption and an increment of pulse over the pre-exercise rate of 60% of the difference between maximal and pre-exercise rate. All exercise was done with continous electrocardiography under the supervision of a nurse. This exercise was done 3 times a week at a pulse rate from 114-130 and 160-170.

Statistical analysis was by linear regression (25) and by the binomial test (26).

RESULTS

The volunteers felt well during the study; indices of health and nutritional status confirmed their well being. Vital signs were stable; the coefficients of variation of 44 biweekly weights were 0.78 and 0.57%.

Table II shows the absence of hematological change during depletion. None of the tabulated characteristics regressed significantly versus time of depletion. All values for ceruloplasmin and superoxide dismutase activity were within the 95% confidence limits for male members of the Center staff. The low values for plasma copper, 64 and 70 µg/dl, were more than three and two standard deviations, respectively, below our normal mean value of 90 µg/dl. Changes in cholesterol were not consistent between volunteers; no hyperlipidemia was found.

Table II. Laboratory Indices During Experimental Copper Depletion

	2082			2083		
	Initial	Lowest	N	Initial	Lowest	N
Hemoglobin (g/dl)	16.6	16.1	5	16.5	15.6	4
Hematocrit (vol.%)	44	43	5	42	42	4
RBC ($10^6/mm^3$)	5.01	4.98	5	5.02	4.84	4
MCV (μm^3)	88	84	5	84	84	4
MCH (pg)	33	32	5	33	31	4
MCHC (%)	38	35	5	39	36	4
Neutrophil count ($10^3/mm^3$)	3.10	2.70	3	2.86	2.58	2
Plasma Iron (g/dl)	151	110	5	107	90	3
Transferrin (mg/dl)	290	290	4	330	255	3
Plasma Copper (g/dl)	80	64	16	83	70	11
Ceruloplasmin	40	26	9	34	24	7
Superoxide Dismutase (U/g Hb)	4293	2899	17	3183	2736	12
Total Cholesterol (mg/dl)	162	205*	16	191	212*	11
HDL Cholesterol (mg/dl)	41	53*	8	37	56*	8

Linear regression revealed no significant trends consistent with copper depletion of these characteristics with time.
*These are the highest values during the depletion phase as cholesterol is expected to increase during copper depletion.

Figure 1 shows the results of the glucose tolerance tests. Every point after infusion on the curves at the end of depletion was higher than the corresponding point at the end of the control period ($p < 0.008$, n=7, by binomial test). The mean increment in glucose at all points was 38 mg/dl. On repletion the mean decrement was 24 mg/dl. Because the third point on the repletion curve for the second subject exceeded that on the depletion curve by 2 mg/dl, the binomial probability was increased to 0.055. (The probability of 13 decrements out of 14 being due to chance is 0.0009). Some of the points fell below control values on repletion. Fasting glucose and k values (20) for glucose clearance (1.71 to 3.41 mg%/min) were normal. A diet low in copper fed to two other, apparently similar men, did not alter glucose metabolism; only four of fourteen points on the depletion curves exceeded those on the control curves ($P = 0.061$).

For the first volunteer insulin declined at only five of the seven points (not significant) during depletion and declined further at all points during repletion ($p < 0.008$). Insulin of the second volunteer declined at six of seven points in depletion and in repletion ($p = 0.055$). All insulin values in repletion were lower than during the control period ($p < 0.0001$) for both volunteers. The mean concentration of insulin for both volunteers at each point after infusion declined by 10 μU/ml during depletion and declined further by 25 μU/ml during repletion. Table III shows the combined insulin data on both volunteers.

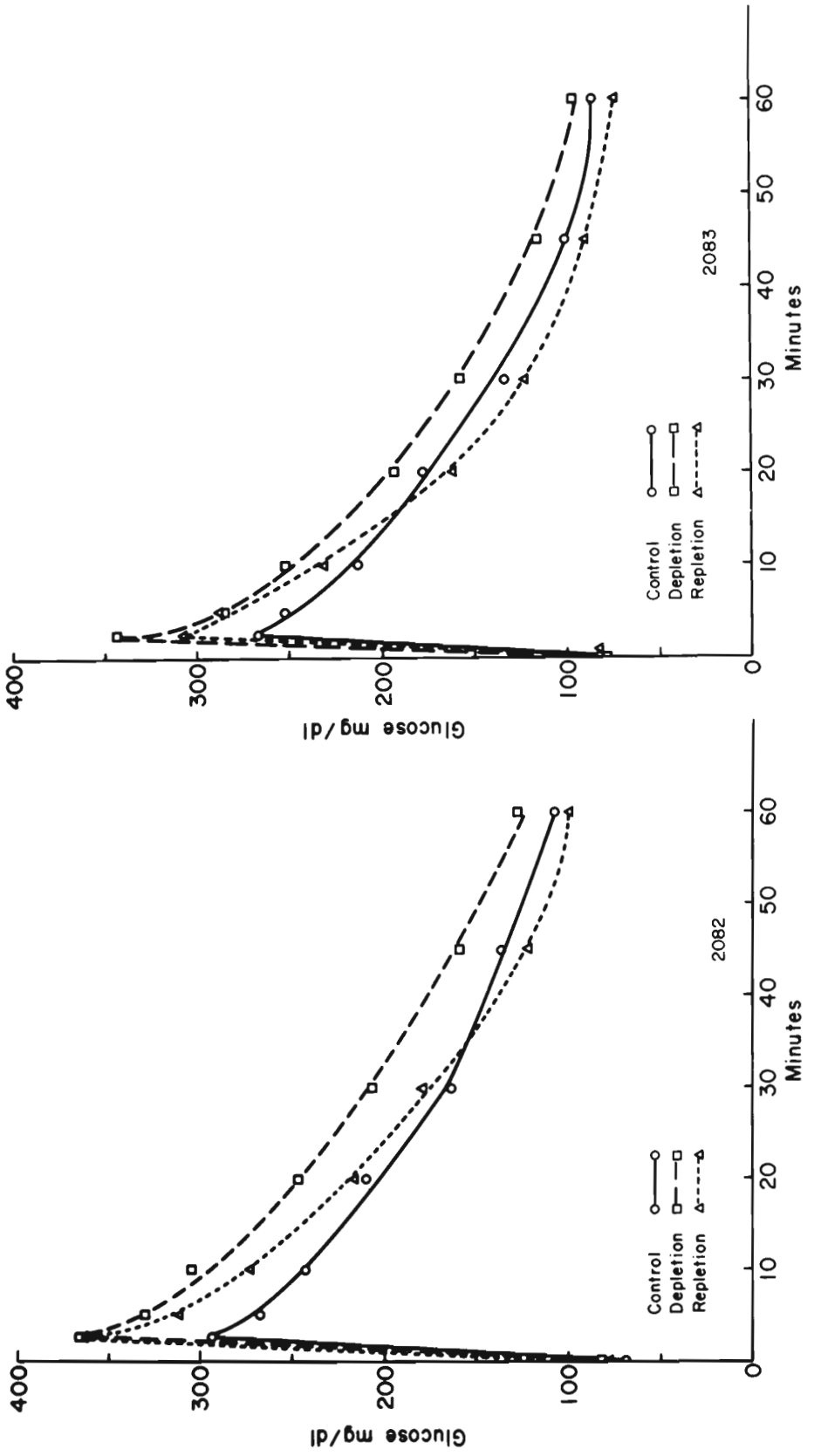

Fig. 1. Response to intravenous glucose (see text).

Table III. Mean Plasma Insulin for Both Men (μU/ml)

Time, Min.	Control	Depletion	Repletion
0	31	12	11
2 1/2	100	99	87
5	98	95	64
10	88	72	42
20	76	80	36
30	66	56	24
45	65	41	19
60	46	24	17

Mean coefficient of variation for all values was 23%

DISCUSSION

The mean daily intake of copper during the depletion phase of the study was 0.78 mg. This amount was less than the 2 mg generally thought to be required to compensate for daily urinary and fecal loss (27) and is less than these losses (mean 1.30 mg/day) by other men studied under similar environmental conditions (15).

Since copper was shown to be essential for mammals (28), anemia has been considered to be the sine qua non of deficiency. However, recent experiments with animals demonstrated copper deficiency without anemia (29). None of the standard hematological data from the two volunteers is indicative of anemia. The mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration seem a bit high; one expects them to be low in the anemia of copper deficiency (30,31). Plasma copper fluctuated during depletion and did not regress significantly versus time. High and low values were found both early and late in depletion. The lowest values for plasma copper for each volunteer were outside the normal range; these values can be interpreted as evidence of mild depletion of copper. Hypocupremia is the first detectable sign of copper deficiency in swine (31); in infants, plasma copper is more sensitive to copper depletion than are either neutropenia or anemia (32). Thus, it appears that the dietary copper requirement of the two men exceeded 0.78 mg per day.

Decreased clearance of glucose in depletion and increased clearance in repletion is consonant with experiments with animals (11-13). In rats (29,33) plasma cholesterol is more sensitive to copper depletion than is hematocrit. Perhaps, under some circumstances, decreased glucose tolerance is a more sensitive index of copper nutriture than is either standard hematology or plasma copper, ceruloplasmin or cholesterol, and superoxide dismutase. This interpretation and these data require confirmation.

The mechanism by which copper deficiency or copper depletion altered the metabolism of glucose in these volunteers is obscure. The k value for each volunteer was lowest in the control phase and highest in the repletion phase of the study: 1.71, 1.81, 2.21 and 2.31, 2.43, 3.41, respectively. The decreased glucose clearance

during depletion may be explained partially by the slightly lower concentrations of insulin in plasma. However, increased glucose clearance in repletion was accompanied by a further decline in circulating insulin. The decline in insulin in repletion seemed to be most pronounced in the fasting state and later during the glucose tolerance tests; i.e., when blood sugar was less than 200 mg/dl. It appeared as if the insulin was more effective in repletion.

Rats deficient in copper sometimes have lower fasting glucose concentrations (12, and perhaps 13); however their clearance of oral (12) and intravenous (13) glucose is impaired. This abnormality seems to be reflected in increased glycosylation of hemoglobin (14). Generally, deficient rats have lower circulating insulin (12,13); however, Hassel et al. (13) found a biphasic insulin response to glucose load with low fasting concentrations early during the glucose tolerance test and higher concentrations later.

Copper seems to enhance the activity of insulin or to mimic its action when studied in vitro. Copper salts were found by Saggerson et al. (34) to be more effective than salts of several transition elements in inhibiting lipolysis of fat cells stimulated by adrenaline or glucagon and in stimulating their lipogenesis. The concentration (34) of copper available to the cells probably was considerably less than that stated (2 mM) because of the albumin in the buffered medium. Fields et al. (35) found that the incorporation of radioactive glucose into carbon dioxide or lipid by adipocytes stimulated with insulin was greater if the cells were obtained from rats supplemented with copper than if the cells were from rats deficient in copper. Adipocytes from rats deficient in copper bound less insulin than cells from rats supplemented with copper (35). This decreased binding was due to a decrease in receptor number.

Rats injected with streptozotocin become hyperglycemic. Control of this hyperglycemia by insulin in rats deficient in copper can be improved if insulin and cupric chloride are injected together (36). Insulin plus copper produced a greater incorporation of glucose into the lipids of epididymal fat pad than that produced by insulin alone (36).

The activity of lipoprotein lipase is decreased in rats deficient in copper (37). This decrease may be the result of the altered metabolism of insulin and glucose in copper deficiency because this enzyme is synthesized in response to insulin and glucose (38). If the decreased enzyme activity results only from lack of a metal-protein complex (37), the decrease in enzyme activity could alter the metabolism of insulin because ingestion of fat or infusion of fatty acids can stimulate insulin secretion (39,40). Rats deficient in copper are hypertriglyceridemic (41). However, if alterations in lipid metabolism produced alterations in insulin utilization in these volunteers, the effects were subtle because neither volunteer responded to copper depletion with significant hypertriglyceridemia.

Copper deficiency also produces anatomical changes in the pancreas (42,43). The effect appeared to be confined to the exocrine part of the gland and was described (43) as loss and atrophy of acinar cells, with hypertrophy and degeneration of mitochondria. Disorganization and necrosis of acinar cells also was found. Although the islets appeared normal on microscopy (42,43), the release of insulin may have been impaired (12,13).

Thus although measurements of insulin in these volunteers did not account for all the changes in glucose tolerance during repletion, experiments with animals reveal several ways in which copper could modify glucose metabolism. The effect could have been mediated via insulin receptors, the binding of insulin to cells or, indirectly via lipid metabolism.

The amount of copper in the diet (0.78 mg/day) was not uniquely low. Probably less than 25% of diets contain the 2 mg of copper thought to be required each day by adults (27) to compensate for urinary and fecal loss. According to our recent data (15,43) some adults may require more than 2 mg of copper daily. Numerous articles on copper in contemporary diets have been reviewed (45); four are among the more accessible (46-49). The geometric mean of copper in 20 diets (47) was 0.82 mg. This value is between the mean of 1.0 mg/day (46) and 0.7 mg/day (48,49) which define approximately the lower half and the lowest 16% of the frequency distributions. Approximately one third of the daily diets in one of these studies (46) contained less than 0.8 mg of copper per day (W.R. Wolf, personal communication). Copper in these diets was measured by atomic absorption spectrometry (46-49).

It seems unlikely that the volunteers in this study are unique in their response to dietary copper; although two other men studied under similar conditions did not respond to a diet low in copper with a change in glucose clearance. Children with Menkes' disease, an hereditary inability to absorb dietary copper (50), are glucose intolerant (51). Diets containing an amount of copper similar to that which produced the change in glucose clearance seem to be rather common. Animals deprived of copper respond similarly to these men.

People with idiopathic diabetes mellitus of the less severe form (type II, adult onset, or insulin resistant diabetes) and people with glucose intolerance of insufficient severity to be called diabetics are common. These conditions have an adverse effect on life expectancy. Perhaps suboptimal copper nutriture contributes to these conditions.

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